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CROONIAN LECTURE

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[Plates 1–9]

The problem to be discussed was presented as long ago as 1918 when, at the request of the Medical Research Committee (now Council), I was engaged on an investigation to discover the cause of rickets. It was noticed that some of the experimental animals became very incoordinated in their movements and that this disability might develop with or without rickets. The independence of the two syndromes thus experimentally produced and the fact that incoordination of movement does not form a part of the usual clinical picture in rachitic children suggested that the cause of the incoordination in the animals was independent of but related to that of rickets. The main result of the earlier work was to establish the fact that rickets was due primarily to a deficiency in the diet of an antirachitic vitamin which was fat-soluble and was either vitamin A or a substance closely allied to it in properties and distribution (1919, 1921).

At that time vitamin A, discovered 5 years earlier by McCollum & Davis (1913), was the only recognized fat-soluble vitamin. But little was known of its chemistry in 1918 and it was identified by two biological tests: (1) its power to promote growth in young rats when added to diets previously deficient in it, (2) the fact that when it was absent from the diet animals developed xerophthalmia, which could then be cured if substances containing it were administered. It was found later that the vitamin A of those days was really a mixture of two fat-soluble vitamins—one of which retained the designation vitamin A and the second, the calcifying or antirachitic vitamin, was called vitamin D.

Looking back, it is of interest to note that, of the two biological properties of the original vitamin A complex—growth promotion and antixerophthalmic action in rats—the former was due more to its vitamin D than to the vitamin A moiety. It is true that the absence of vitamin A, as we now know it, causes young rats to stop growing, but this is largely because, unlike other young experimental animals, rats develop infective foci in many places—middle ear, kidney, lung, mouth, indeed almost anywhere—and they stop growing primarily for this reason. Vitamin A prevents or often cures these infections and thereby allows growth to proceed. But vitamin D, the other part of the original vitamin A complex, is a real growth promoter in the sense that, in its absence, bones cease to grow normally in length because calcium metabolism, including that at the bony epiphyses, stops functioning. Thus, there was no more reason why the name vitamin A should not have

been given to what is now known as vitamin D—the real growth promoter—than that it should have been retained to designate the antixerophthalmic entity.

The differentiation of the vitamin A complex into its constituents required the labour of a large number of investigators over several years, and it was not until after 1924 that it became possible to give the calcifying vitamin, i.e. vitamin D, to animals without also including the antixerophthalmic vitamin A. This could be done because in that year Steenbock & Black (1924) published their work showing that vegetable oils, devoid of fat-soluble vitamins, acquired the calcifying properties of vitamin D when exposed to ultra-violet radiation, an extension of Huldschinsky's (1919, 1920) discovery that children could be cured of rickets by similar exposure of their skin. This aspect of the subject was developed by the joint announcement of Rosenheim & Webster (1927), and of Windaus & Hess (1927), that ergosterol was the pro-vitamin D in fats, and that this substance could be converted into the vitamin by ultra-violet radiations. This story ended when pure vitamin D (calciferol) was prepared by Bourdillon and his colleagues (Askew, Bourdillon, Bruce, Jenkins & Webster 1930).

As regards vitamin A, it became possible to examine more closely the nutritional properties of this vitamin when Euler and his colleagues (Euler, Euler & Hellström 1928) found that carotene had the biological properties of vitamin A. Shortly after Karrer had obtained a highly concentrated preparation of vitamin A and determined its structure (Karrer, Morf & Schöpp 1931), the effects of adding this substance in pure form to synthetic diets could be studied in greater detail.

About 1926 interest in the problem of incoordination in animals was renewed (Mellanby 1926), and it was ultimately established (Mellanby 1930) that a deficiency of vitamin A in the diet was the determining condition and that the other part of the original vitamin A complex, namely vitamin D, was not directly concerned with the aetiology of this disability.

The problem of incoordination

(In the lecture a cinematograph film was shown to demonstrate the problem to be studied.)

Young animals, including puppies, rabbits and rats, become incoordinate when vitamin A and carotene are deficient in their diets. The basal diets used to produce the abnormal behaviour in the three types of animals are as follows:

Daily rations

Dog:	Cereal (flour, oatmeal, etc.)	80–200 g.
	Lean meat	15–25 g.
	Peanut oil	10 c.c.
	Separated milk powder	20–25 g.
	Baker's yeast	5 % of cereal
	(or dried yeast	$1\frac{1}{4}$ % of cereal)
	NaCl	1 % of cereal
	Ascorbic acid	5 mg.
	Vitamin D_2	1000–2000 i.u.

Daily rations (continued)

Rabbit:	Oats and bran in proportion of 4:1	30–60 g.	
	Alfalfa leaf, heated and oxygenated to destroy carotene	10 g.	
	Baker's yeast	5 % of oats and bran	
	Ascorbic acid	0·5 mg.	
	Vitamin D_2	200 i.u.	
Rat:	Oatmeal	89 g.	10–15 g. of this diet daily
	Alfalfa leaf, heated and oxygenated to destroy carotene	2 g.	
	Peanut oil	0·5 c.c.	
	Calcium carbonate	Various amounts	
	Sodium pyrophosphate	1·71 g.	
	Baker's yeast	10 % of stock diet	
	NaCl	0·85 g.	
	Ascorbic acid	0·5 mg.	
	Vitamin D_2	125 i.u. daily	

If vitamin A or carotene be added in sufficient amount to these diets, the animals remain normal. Vitamin A can be added in pure form as the naphthoate or acetate, or in the form of foodstuffs containing this vitamin, such as mammalian or fish-liver oil or butter: β-carotene can be added as a pure substance or in carrots or green vegetables.

If the vitamin A-deficient diet be given to puppies 6–8 weeks old, incoordination of movement usually begins in 2–3 months and may be very severe in about 5 months. The rate of development of the condition and its severity depend on the time taken for the stores of vitamin A, especially those in the liver, to disappear.

The behaviour of the affected animals varies somewhat from animal to animal, but generally they are unable to run straight and they may lurch from side to side or even fall over. Sometimes they circle continuously. They hold their heads in abnormal ways, generally on one side with the nose turned upwards and their ears awry. In puppies it is especially noticeable that they cannot fix their attention for any length of time: unlike normal animals, they will not follow an individual for more than a few seconds but will wander off, lurching about in all directions. In the early stages they may run about sniffing, licking the ground and attempting to eat anything they come across. At a later stage puppies may become quite deaf and sleep a lot, and it may be necessary to touch them in order to draw their attention even to the presence of food. Usually their appetite remains good and they increase in weight at about the same rate as control animals eating food of the same composition but with the addition of vitamin A. Although the incoordination may be great, the animals often move about vigorously in the earlier period of A deficiency. Later, stiffness, especially of the hind legs, is apparent and their activity is greatly reduced and, occasionally, in a longer experiment the animals stand or lie about. To what extent this is due to loss of vision, hearing, and sense of position on the one hand, or to loss of muscle function on the other, is not clear, but probably both are involved. A vitamin A-deficient rabbit moves its head

in a curious way as if it did not know its position in relation to the rest of the body: sometimes the head is moved up and down and sometimes from side to side, and when at rest is often askew.

Two other points about the development of incoordination must be mentioned. The first is that young animals are much more quickly and intensely affected by vitamin A deficiency than older animals. Whereas incoordination develops in 2–5 months when the diet begins at 6–8 weeks of age, it may only appear after 8–12 months in dogs beginning the defective diet at the age of 4–6 months. In adult animals, say $2\frac{1}{2}$ years old, the development of incoordination may only appear after 15 months or more of the defective diet, and then it is never so severe as in young animals. The second point of interest is that, if potato replaces the cereal (bread or oatmeal) in the vitamin A-deficient diets, then in my experience it is difficult to produce incoordination to more than a slight degree. It is true that potato contains a trace of carotene, but so also do bread of 85 % extracted flour and oatmeal. It looks as if potato either contains some protective substance other than vitamin A or carotene which acts like vitamin A, or it does not make the same call on vitamin A reserves as is made by bread. It may be added that, even when the livers of puppies receiving potato are found after death to be devoid of vitamin A, the animals are remarkably free from the incoordination that develops in those eating cereals.

Amount of vitamin A necessary to prevent abnormality

Before continuing the story of the investigation, it might be well to give some idea of the quantities of vitamin A which can prevent the abnormal changes in puppies. 100 i.u. of vitamin A per day are too small a dose to prevent all the incoordination changes. 1000 i.u. give complete protection. Let it be assumed that 500 i.u. would be sufficient. Now 5 i.u. of vitamin A weigh 1 γ, so that 500 i.u. weigh $100\,\gamma = \frac{1}{10}$ mg. The average daily intake of food of an experimental puppy weighs, when dry, about 150 g. Thus one part of vitamin A in about $1\frac{1}{2}$ million parts of food is sufficient to prevent all defect. It will be realized from these figures how very powerful is this substance in directing proper development and function of young animals.

The investigation

Lesions of the peripheral and central nervous system in vitamin A-deficient animals

(*a*) *Peripheral nerves*

It was not unnatural that attention should be first directed to examination of the nervous system of these animals, and, in view of the general incoordination and especially of the head movement, that the vestibular division of the 8th nerve was an early objective. Examination by Marchi's method and later by other methods soon revealed the degenerated condition of many of the nerve fibres in

– A animals.* It was more surprising, however, to find that the cochlear division of the 8th nerve was even more affected than the vestibular division, for, at that time, it had not been noticed that these animals were often deaf. Further examination of the cranial nerves showed that the optic and trigeminal nerves also degenerated. The investigation was rather side-tracked at this stage, because it seemed of interest to see what, if any, was the relation between degeneration of the trigeminal nerve and xerophthalmia. The well-known relation between damage to the Gasserian ganglion, particularly following operative removal of the ganglion in trigeminal neuralgia, and keratitis neuroparalytica, suggested a similar or related mechanism in the xerophthalmia of vitamin A deficiency. Another instance which is usually regarded as evidence of neurotrophic control of the 5th nerve over corneal infection is that of herpes zoster ophthalmicus. Of course, the pathological lesions of xerophthalmia, keratitis neuroparalytica and herpes zoster ophthalmicus are not identical but, if xerophthalmia were accompanied by degeneration of the 5th nerve, it might be regarded as evidence that this condition also was possibly neurotrophic in origin. It proved, in fact, that xerophthalmia was nearly always accompanied by 5th nerve degeneration and, so far as the evidence went, there was some support for the view that the eye condition was secondary to the irritative or degenerative changes in the Gasserian ganglion cells of the first division of the 5th nerve (Mellanby 1934*b*).

It was also found that degenerative changes in the cranial nerves were almost entirely confined to afferent fibres, but, even so, some of these escaped destruction, as, for instance, the afferent fibres of the vagus nerve. On the motor side there was little or no degeneration; with very few exceptions all the fibres in the 4th, 6th, 9th, 10th, 11th and 12th were normal, even in badly affected animals. The 3rd and the 7th nerves sometimes contained degenerated fibres in severe cases, but these were never numerous. Again, the 1st and 2nd divisions of the 5th nerve, which are largely sensory, contained many more degenerated fibres than the 3rd division, which is mainly motor in function. This brief survey of the cranial nerves shows that degeneration in – A animals is mainly, though not entirely, confined to the afferent side.

When the examination was extended to the spinal roots it was again found that the posterior (afferent) roots contained many degenerated fibres at a time when no degeneration was seen in the anterior (efferent) roots. Occasionally, however, in long-term experiments a few degenerated fibres were found in the anterior roots. What was particularly perplexing at this stage of the work was the fact that, whereas the posterior root on one side might be degenerated, the corresponding root on the other side might be normal or nearly so. It was also found that the spinal nerves in the cervical region were more degenerated than those of the lumbar, although sometimes the sciatic nerve derived from the latter was much affected.

* For the sake of brevity the terms + A and – A dog (or animal) will be used to denote dogs brought up on diets containing, and those deficient in, but not devoid of, vitamin A respectively.

The dorsal nerves regularly showed least degeneration (Mellanby 1931, 1933, 1934, 1935).

(*b*) *Peripheral ganglia*

The condition of the peripheral ganglion cells was in accordance with the distribution of the degeneration in the peripheral nerves described above. For instance, the cells of the ganglion cell layer of the retina showed varying degrees of degeneration from chromatolysis and eccentricity of the nuclei to complete breakdown and disappearance of the cells. Those cells which were farthest away from the optic disk were liable to be earlier affected than the nearer ones. In one dog whose diet had been A deficient for several years, all the ganglion cells of the retina had disappeared and the dog was completely blind.

The cells of the Gasserian ganglion were also often degenerated, probably earlier even than those of the retina. It was also noticed that these cells were often elongated and misshapen at a stage before degeneration, but the significance of this was not understood at the time. The cell stations of the cochlear and vestibular divisions of the 8th nerve, namely, the spiral and Scarpa's ganglia, were not examined at this stage of the inquiry, but it was found later that both were affected in −A animals, especially the former.

Examination of the cells of the posterior ganglion revealed degeneration, as was expected from the condition of the afferent spinal nerves. Here again the changes were more marked in the cervical region of the cord than in the lumbar and dorsal regions. Cells of the ganglion trunci vagi were intact.

(*c*) *Tracts of the central nervous system*

Having now obtained a view of the peripheral nerve changes, attention was directed to a more detailed examination of the central nervous system. This part of the inquiry was tedious and prolonged. Only a summary of the results need be given here (Mellanby 1930, 1931, 1933, 1935). The nervous systems of puppies, rabbits and rats fed on vitamin A-deficient diets were examined and, although there were differences in the degenerative changes in the various types of animal and even from animal to animal of the same species, there was general agreement in the picture.

The following is a survey of the distribution of the degenerated fibres in the rabbit:

(1) The maximum amount of degeneration was found in the central nervous system between the pons and the lower cervical cord. Below the cervical region of the cord the amount of degeneration diminished greatly, and above the pons there was hardly any degeneration.

(2) The ascending fibres of the cord were more affected than the descending. In particular, the descending fibres having their origin in the cerebral cortex, i.e. the pyramidal tracts, escaped destruction.

(3) Of the primary ascending neurons in the cord the greatest degeneration was seen in the cervical region, and it was here that the posterior columns of Goll and

Burdach were most affected. In the case of secondary ascending neurons, the ventral and dorsal cerebellar tracts (indirect and direct cerebellar) were most degenerated; indeed, their degeneration was usually obvious even in the lumbar region but increased greatly in passing up the cord. Degeneration of the cerebellar fibres could be easily traced up to the cerebellum—the ventral (indirect) cerebellar tract entering the cerebellum through the superior cerebellar peduncle and the dorsal cerebellar through the restiform body and the inferior cerebellar peduncle.

Many degenerated fibres were also found in the anterior and antero-lateral columns of the cord. Some of these were ascending and included fibres of the spino-reticulo-thalamic and spino-tectal tracts.

Although the pyramidal tract fibres were not affected, this does not mean that all descending fibres escaped destruction. Degeneration was found, for instance, in the posterior longitudinal bundle, in the rubro-spinal tract, and in the vestibulo-spinal tract. It will be noticed that all the descending tracts which suffered change had their origin in or below the mid-brain.

(d) Nerve cells of the central nervous system

Examination was then made of the groups of cells in the central nervous system and, although this was by no means complete, a general picture of the effect of vitamin A deficiency on these cells was obtained. As was expected, the pyramidal motor cells in the cerebral cortex and usually the motor anterior horn cells of the spinal column were normal even in animals which were badly affected by the deficiency. On the other hand, Clarke's column cells giving rise to the cerebellar tracts were often very abnormal in form and many were destroyed. Towards the lower medullary level, cells of the cuneate and gracile nuclei, relay stations of the posterior column fibres, showed much degeneration.

Other nerve cells which are liable to degenerate in $-$A animals include those of nucleus solitarius, nucleus ambiguus, vestibular nuclei, nucleus of the descending branch of the 5th nerve, the red nucleus, Purkinje cells in the cerebellum and the dentate nucleus.

This brief survey of the distribution of degenerated nerve fibres and cells of the central nervous system in $-$A animals shows clearly that no sharply defined group of neurons was affected. Although at the time it was thought that the nerve degeneration was directly determined by a toxic condition due to the deficiency of vitamin A, the explanation seemed to be both unsatisfactory in itself and difficult either to prove or develop. Such a hypothesis assumed that a special chemical or enzymic reaction was necessary for the maintenance of structure and function of particular groups of nerve cells which seemed to bear no relation to one another, while other groups were independent of this reaction. For instance, what possible metabolic factor could account for:

(1) the large amount of degeneration between the level of the entrance of the 5th cranial nerve and the lower cervical region of the cord and the usually normal appearance of the higher parts of the brain;

(2) not only the degeneration of the primary ascending neurons but also of secondary ascending neurons, e.g. the cerebellar tracts and the fillet;

(3) the limitation of the degeneration of the descending fibres to those starting in and below the mid-brain;

(4) the uneven distribution of degeneration on the two sides of the body, especially in the posterior spinal roots, which sometimes occurred?

No obvious method of investigating such a hypothetical and specialized chemical mechanism offered itself. Consequently, the work was at this stage put in the background for a number of years.

A new line of attack

A time came when the unsatisfactory explanation of the nerve degeneration found in animals brought up on vitamin A-deficient diets impelled a return to the problem.

It was still felt that the vestibule and its nervous connexions held the main secret and, just as the first inquiry was directed to the vestibular division of the 8th nerve, so now it was decided to see exactly what was the state of the vestibule and the internal ear in these affected animals. Preparations of the labyrinthine capsules of +A and −A dogs were therefore made and serial sections were cut after decalcification of the bone and embedding in celloidin (Mellanby 1938). Examination of these sections led to the surprising discovery of masses of newly formed bone in the modiolus and internal auditory meatus, which were interfering with and ultimately destroying both the cochlear and vestibular divisions of the 8th nerve (figure 1, plate 1). The amount of bone overgrowth seemed to determine the degree of incoordination and the abnormal behaviour of the dogs. The periosteal bone of the capsule, especially that part adjacent to the brain, also became abnormally thick, so that the helix and vestibule were more deeply placed in the labyrinth, causing an increase in length of the 8th nerve. In severe cases, the internal auditory meatus appeared to be completely blocked by the bone overgrowth, but further examination of serial sections usually revealed that the nerves still had some kind of a passage, although this was twisted and further lengthened. The spiral ganglion of the cochlear nerve began to degenerate early and in severe cases all the cells disappeared. On the other hand, the cells of Scarpa's ganglion, the origin of the vestibular division, were more resistant and were capable of being squeezed into abnormal shapes and yet retaining their minute structure and probably their function. However, even in this case there was a limit to their resistance and many cells were ultimately completely destroyed.

This observation of the relative stability of the cochlear and vestibular divisions of the 8th nerve to mechanical injury is an established fact, well known to otologists. The cochlear nerve resembles the optic nerve in that injury to any part of the neuron destroys it entirely. The vestibular nerve, however, reacts like the majority of other nerves and follows the Wallerian law of degeneration, i.e. the nerve degenerates from the point of injury peripherally to the nucleus.

It was further found that, in addition to the bone overgrowth and the nerve degeneration, the cochleas of severely affected −A animals developed a serous labyrinthitis with albuminoid coagulation of the perilymph. This condition had probably no relationship to the nerve destruction but, as will be seen later, might be determined by changes in the cerebro-spinal fluid of the subarachnoid space in the posterior fossa with which the perilymph communicates by means of the cochlear aqueduct. It is probable that the labyrinthitis destroyed the organ of Corti, as it is known to do in man. Degeneration of the cochlear nerve did not in itself bring about the destruction of this organ, and in one experiment, in which vitamin A was given after the cochlear nerve had degenerated, serous labyrinthitis was absent and the organ of Corti remained intact, while that of a litter mate which did not receive the vitamin degenerated completely in the presence of serous labyrinthitis.

It was thus clear that a new condition of prime significance, namely bone overgrowth and dysplasia, had presented itself as a possible intermediate factor between the widespread nerve degeneration and the deficiency of vitamin A and carotene in the diets of these animals. It was now necessary to see whether the same phenomenon accounted for the nerve degeneration described above in other situations.

Bone overgrowth in vitamin A-deficient animals in relation to the nervous system

Naked eye examination of the bones surrounding the other cranial nerves showed at once that abnormal bone growth and overgrowth in vitamin A-deficient animals were by no means confined to the internal auditory meatus. There was great thickening of many skull bones, especially of the petrous ridge, the enlarged bone of which clearly nipped the 5th nerve. Farther forward in the base of the skull the optic nerve was caught tightly between the thickened sphenoid bone and the dura mater. Following these superficial examinations it was decided to prepare serial sections of all the cranial nerves and the surrounding bones and to examine the whole course of each nerve (Mellanby 1943). Some of the results will now be briefly described, in order to show that bone dysplasia can be regarded as the main cause of the extensive nerve degeneration found in −A animals.

Olfactory nerve

In these animals the cribriform plate may be greatly thickened and the marrow spaces therein may be enlarged and full of fat. This general thickening of the bone is associated with a narrowing of the channels through which the nerves pass and the nipping of the nerve bundles, as can readily be seen by the examination of serial sections. Under higher powers of the microscope evidence of this nipping is shown by the fact that the nuclei of the nerve sheaths are crowded together in those parts of the nerve within the cribriform plate, but not on either side of it. Also it is found that there are fewer nerves in each bundle than in the normal animal and, besides being squeezed, they are twisted in their passage. It is difficult to say

to what extent the nerve fibres are destroyed. They are certainly not all destroyed; on the other hand, the relatively small number passing across the subarachnoid space of the olfactory lobe, as compared with those of normal animals, indicates a large amount of destruction in severely affected animals. It will be remembered that $-A$ puppies often run about sniffing vigorously; this may indicate their effort to counterbalance the loss of much of their sense of smell because of nerve degeneration.

Optic nerve

Serial sections of this nerve and the surrounding bone from the retina to the optic thalamus in $-A$ animals show that the nerve is subjected to pressure and distortion in some parts of its course owing to abnormal and excessive bone growth. The optic canal is longer and is more of a real canal than in the normal animal. After emerging from the canal on to the base of the skull, the nerve passes through a sharper and deeper groove than that normally found and its cross section, instead of being round or oval, is triangular. Part of the distortion seems to be due to the squeezing of the nerve by the thickened dura mater. The bone pressure is not as severe as that found in the case of the trigeminal and auditory nerves but, on the other hand, the optic nerve is much less resistant to mechanical pressure than most nerves. Even so, severe degeneration is ultimately produced. An added factor, probably of great importance in the degeneration of the optic nerve, is the increased intracranial pressure found in $-A$ animals. The importance of this factor has been mentioned in relation to the papilloedema which develops in the optic disk and is one of the reasons for the ultimate blindness of some of these animals.

It is of interest to note that blindness of nutritional origin in young cattle has been known to agricultural scientists for some years. Work on this subject was published by Moore, Huffman & Duncan (1935) who said that the optic foramen of affected calves was improperly developed. Although at the time they did not think that carotene deficiency was responsible for this defect, it was proved later by Moore (1939) as the result of a further investigation that this was indeed the case and that these defects in calves could be prevented by foods containing carotene. There can be but little doubt that this nutritional defect of vision is the same as that now being discussed in the experimental puppies, and further examination will probably reveal many other nerve lesions in these calves.

Trigeminal nerve

This nerve is much affected by bone overgrowth in $-A$ animals. The bone hypertrophy is especially large about the petrous ridge, and sections through the level of the Gasserian ganglion show it to be squeezed and the nerve connecting it with the central nervous system to be compressed and twisted at a right angle towards the midline, instead of passing directly into the pons. The great amount of destruction of nerve fibres in the 1st and 2nd divisions of the 5th nerve, referred to

previously, can now be explained by the pressure effect of the hypertrophied bone on their cells of origin within the Gasserian ganglion. The relatively slight effect on the mandibular or 3rd division is no doubt due to the much larger number of motor fibres in this branch, these fibres having their cells of origin inside the brain and away from the compressed Gasserian ganglion.

The other cranial nerves

As previously mentioned, it is rare to find much degeneration in the 3rd, 4th, 6th, 7th, 9th, 10th, 11th, and 12th nerves. In view of the present knowledge of bone dysplasia in −A animals, this can probably be explained by the fact that, being mostly motor nerves, their ganglionic origins are inside the central nervous system and are not directly subjected to bone pressure. Where they have peripheral ganglia—as in the case of the jugular ganglion, the ganglion trunci vagi and the geniculate ganglion—the nerve cells generally escape degenerative changes, although the few degenerated fibres occasionally found may have their origin in these peripheral ganglia. Of these nerves, the one most affected by pressure is the 7th. Both in its passage with the 8th nerve through the internal auditory meatus and in the facial canal, bone overgrowth sometimes presses heavily on the 7th nerve and may twist and squeeze it to a ribbon. In spite of this, however, it is rare to find more than a few degenerated fibres, and it would appear as if motor nerves in general are very resistant to mechanical pressure.

The 9th, 10th and 11th nerves are not much compressed by overgrown bone, although the bones through which they pass are greatly hypertrophied. Their escape may be due to the fact that the jugular canal, through which they pass, is bounded by the temporal and occipital bones, and that a canal of this nature is not so much narrowed by bone intrusion as if it were in one bone. It would appear also as if the bone hyperplasia in this position were rather at the expense of the tympanic cavity than at that of the jugular canal. Together with the 12th nerve, the 9th, 10th and 11th nerves are increased in length by having to traverse the thickened bone, but it is possible that they grow naturally with increased distance and may not be subjected to any stretching or increase of tension. In any case they seldom contain any degenerated nerve fibres even in severely affected animals.

Spinal nerves

It was clearly necessary to see whether bone overgrowth and dysplasia could also account for the degeneration of the posterior roots of the spinal cord (Mellanby 1941). Serial sections through the vertebrae, especially in the cervical region, soon showed that this was the case. The spinal canal was seen to be smaller than normal, thus allowing less space for the spinal cord. Part of the reduced space was due to the approximation of the body to the lateral portions of the vertebrae; in some cases the space between them was too small for the passage of the spinal nerves and the posterior root ganglia could be seen to be compressed between the bones (figure 2, plate 2). This squeezing of the posterior root ganglion was often greater on one side of the vertebra than on the other at any one level and this explained

what had previously been difficult to understand, namely, that sometimes in less severe experiments degeneration in the posterior roots was present only on one side (figure 2*b*).

It was now clear that the reason given above for the destruction of the 8th nerve in −A animals, i.e. abnormal and increased bone growth, could account for most, if not all, of the degeneration in other peripheral nerves, namely the olfactory, optic, trigeminal and posterior spinal nerves. It was also obvious that degeneration of the first ascending neurons, having their origin in the posterior root ganglion and travelling via the columns of Goll and Burdach in the spinal cord, could be similarly explained.

Attention was again directed to the skull itself, to see what effects bone dysplasia had on the different parts of the brain.

The brain and abnormal growth of the skull bones

Mesial sagittal sections of the skulls of +A and −A litter-mate puppies (figure 3, plate 3), which have grown at equal rates in the experimental period show clearly the main positions of bone dysplasia in the −A animal and their effects on the brain outline. The most hypertrophied bones surround the posterior fossa and include the basi-occipital, supra-occipital and the temporal bones. Passing forward towards the nose, the basi-sphenoid is also seen to be thickened, the sella turcica is deeper than normal due to the overgrowth of the posterior clinoid process, which bends forward and tends to press the pituitary against the basi-sphenoid, the parietal bones are less hypertrophied, and the frontal bones are but little affected.

It is clear that the thickened bones have pressed on and distorted the shape of the brain stem in the posterior fossa. The fourth ventricle is squeezed between the cerebellum and the medulla oblongata. The pons is compressed and the medulla itself is misshapen, developing a waist-like appearance which is absent from the normal medulla. The dorsal surface of the cerebellum is flattened and its posterior end is pushed through the foramen magnum. This reduces the capacity of the cisterna magna, which is also further reduced by the thickened bones of the atlas vertebra (Mellanby 1941).

Intra-cranial pressure and the cerebro-spinal fluid

An increased intra-cranial pressure in −A animals can often be demonstrated directly during the experimental period if a needle is inserted in the cisterna magna and the pressure of the cerebro-spinal fluid is recorded. Frequently this pressure is about twice that in +A litter-mates. At later stages in severe cases, however, records of this kind cannot be taken, partly because it is difficult or impossible to insert the needle into the cisterna owing to abnormal bone growth, and partly because the cerebro-spinal fluid is greatly reduced in amount. This can be readily understood, for pressure on the cerebellum and medulla must occlude partially or completely the 4th ventricle and the foramina of Luschka, so preventing the fluid

secreted by the choroid plexus within the 3rd and lateral ventricles from passing to the subarachnoid space in the posterior fossa and spinal cord. (In the dog there is no foramen of Magendie.) That this is at least part of the explanation of the reduced fluid in the cisterna magna is indicated by the fact that the 3rd and lateral ventricles in −A animals expand and develop a condition of internal hydrocephalus owing to the imprisonment of the cerebro-spinal fluid, which continues to be secreted by the choroid plexus until a limiting pressure is reached. This enlargement of the ventricles is usually not great because the sutures of the skull bones in puppies close comparatively early in life. If, however, the experiments are begun earlier and especially if the mothers get vitamin A-deficient diets during lactation, then a greater internal hydrocephalus can be produced.

In the dog the infundibular cavity is prolonged into the pars nervosa of the pituitary gland, and as it is in direct communication with the 3rd ventricle any increase of pressure within that part of the brain will be communicated to the pituitary. When, therefore, a condition of internal hydrocephalus is present, one would expect distention of the gland. This does occur in −A animals and causes the pituitary to be pressed against the basi-sphenoid and also to be pushed under and against the enlarged posterior clinoid process.

It will be clear now that the observations previously mentioned, namely the development of papilloedema of the retinal disk, of serous labyrinthitis and of compression of the pituitary in −A puppies, can be partly explained by these pressure effects on the brain stem. Papilloedema of the retinal disk is clearly related to the raised intra-cranial pressure. It is true that this pressure is due for the most part to bone changes near the posterior fossa and that the tentorium cerebelli reduces the area for communication of this pressure to the more anterior skull cavity. This is especially so in −A animals in which the tentorium cerebelli has become calcified (see figure 3), but, even so, the increased pressure must be communicated forward through the cerebro-spinal fluid via the aqueduct of Sylvius and doubtless plays a part in the development of papilloedema. The reduced amount of fluid in the subarachnoid space around the brain stem must also affect the perilymph of the internal ear and is probably largely responsible for the production of the albuminoid coagula in the perilymph spaces and the subsequent degeneration of the organ of Corti. The distention of the pituitary body has been referred to above.

We can now also see why the nerve degeneration is particularly great in the pons, medulla and upper cervical region, for it is here that the bone dysplasia and the direct pressure effects on the brain stem are greatest. How these pressure changes bring about the degeneration of wholly endogenous nerve cells and fibres previously described is not easy to understand. It may be due partly to interference with the cerebro-spinal fluid or even with the blood flow. Possibly in some cases degeneration of the secondary neuron may follow that of the primary neuron. Whatever the mode of action, it is evident that the places where the bone is thickest and direct pressure effects on the brain stem are greatest correspond with those parts of the central nervous system where nerve degeneration is most common.

Vitamin A deficiency and bone overgrowth

The investigation had now come full circle. It started by an observation made while studying the aetiology of a bone disease, i.e. rickets. It was now obvious that the incoordination of movement was also primarily due to a bone defect in which some bones grew abnormally, so that, instead of protecting the nervous system, they tended in many places to destroy it.

Now why is it that the bones surrounding the posterior fossa of the cranial cavity should be so much more affected than other skull bones? There is some evidence that those bones which normally grow more vigorously during the experimental period show the greatest hypertrophy. This association between hypertrophy and rate of growth would be expected from the relative rapidity with which the abnormalities described can be produced in young growing animals as compared with adult animals.

Some evidence was obtained that in puppies the bones of the posterior fossa do grow more rapidly during the experimental period than other bones of the skull. If these bones grow more rapidly than other skull bones between the 6th week and the 7th month of life (this includes the usual experimental period), it would be expected that the cerebellum, pons and medulla, which they surround, would grow relatively more than the rest of the brain. This seems to be the case. Taking the average weight of these parts of the brain to the total brain weight at different ages in a number of dogs, it was found that the cerebellum, pons and medulla formed about 13 % of the whole brain weight at 6 weeks of age, 15 % at 22 weeks, and over 17 % at 34 weeks. There is therefore evidence that there is a relatively greater increase in size of the hind brain during the experimental period and therefore, it may be supposed, of its bony covering.

The general effect of A deficiency on bone growth can perhaps best be seen in the case of bones of complicated structure such as the vertebrae, especially those of the cervical region. Photographs of the atlas and axis vertebrae in +A and −A animals are shown in figure 4 (plate 4). These bones in −A animals lose their delicate appearance and become coarse in outline. Sharp edges become blunted and curved surfaces in general become flattened. Their overall size is about the same as that of the vertebrae of the control +A animal, but the constituent parts are larger and thicker at the expense of the central canal through which the cord passes.

This thickening and coarsening of bone may also affect some of the bones of the face, in particular the mandible, the malar bone and the zygomatic process of the temporal bone. Figure 5 (plate 5) shows the malar bones of a normal and a vitamin A-deficient dog. It will be seen that in the latter the bone extends upwards and impinges to a larger degree on the lower margin of the orbit and tends to push it upwards. This swelling below the orbit may give a −A dog a somewhat characteristic facial appearance, as it produces an impression of a shorter nose, although this is not always the case.

The mechanism of action of vitamin A deficiency on bone growth

It is now necessary to make a closer study of the bone changes and see how the distorted growth is produced. In the lantern slides shown in the lecture, it will have been seen that the overgrown bone is of cancellous structure and that the interstices are full of fatty marrow. Generally the increase in cancellous bone is accompanied by a decrease in compact bone. The minute structure of the bone itself is not far removed from normal in structure, and there is no osteoid tissue, as in rachitic bones, nor is there any inflammatory reaction. The presence of osteoid tissue, of course, would not be expected as the diets are rich in vitamin D. It is evident that a larger bulk of bone is laid down in certain positions with a given supply of calcium in the basal diet, but that the total amount of calcium salts is about the same in any bone in both the +A and −A animals. It did not seem probable that phosphorus was a limiting factor, since the diets were adequate in this respect. On the other hand, if the calcium supplied was not sufficient for optimal calcification in −A animals, it might in some way be responsible for the actual bone hypertrophy. It seemed possible that the cancellous overgrowth in animals receiving low calcium without vitamin A indicated an effort by bone osteoclasts to supply the necessary salts to harden the additional new bone laid down by the active osteoblasts: also that, if abundant calcium salts were supplied, bone overgrowth would be prevented. Experiments were therefore made to see what would happen if the calcium intake were increased in both +A and −A dogs. The result of one such experiment on the structure of the cribriform plate is shown in figure 6 (plate 6). It will be seen that the effect of adding extra calcium in the form of calcium carbonate to the diet has been to make the cribriform plate in both the +A and −A dog more compact and less cancellous. On the other hand, the plate in the −A dog is still much thicker than in the corresponding +A dog. In other words, calcium is not the controlling factor as regards bone hypertrophy, but a larger supply than that of the basal diet does influence the bone structure and makes the bone more compact.

Having seen the secondary part played by calcium in this phenomenon, it is now necessary to consider how the bone cells—osteoblasts and osteoclasts—behave under the condition of vitamin A and carotene deficiency. There are obviously two actions to be considered. In the first place, there is certainly an actual laying down of superfluous bone in some parts, due to excessive osteoblastic activity. This is clear in the case, for instance, of the internal auditory meatus (figure 1). Bone is not normally found within this structure, but in the −A animals it may almost occlude the meatus. Similarly, the folded bones that can be seen round the paraflocculus, second, fifth and other foramina indicate excessive laying down of new bone.

On the other hand, all the extra bone found in −A animals cannot be accounted for so simply. In some cases it is clear that the mechanism at fault is not so much bone overgrowth but absence or diminution of absorption of older formed bone,

In figure 3, for instance, it will be noticed that the distance from the outer side of the supra-occipital to the lower margin of the basi-occipital, SB, $S'B'$, is practically the same in + A and − A dogs. The difference is not in the outside measurement but the inside measurement, i.e. from the lower margin of the supra-occipital to the upper margin of the basi-occipital, PO, $P'O'$. The thickening in this position is probably largely due to decreased absorption of the cranial or inner surface of the occipital bone and not so much to the excessive laying down of new bone on its outer surfaces. The same applies to the smaller spinal canal of the vertebrae in the − A animals (figures 2 and 4). This can be explained on the hypothesis that, while the vertebrae grow at least as actively as those of + A dogs on the outer margins, there is a diminished and irregular absorption of the bone lining the vertebral canal. The result here is, as in the case of the bones of the posterior fossa, that the bones grow but the space they surround does not increase sufficiently to allow the growing nervous system to accommodate itself comfortably. The central nervous system and its membranous coverings are thus compressed by the bones and the nervous tissue is ultimately damaged. If these suggestions are correct, it ought to be possible to demonstrate their truth, because the function of the osteoclasts is to remove bone and therefore it would be expected that there would be a difference between the number and activity of osteoclasts on the inner side of these bones, i.e. the side adjacent to the central nervous system, in + A and − A dogs. An examination of the occipital and vertebral bones of these experimental animals was therefore made to detect any such difference, and it was found that this indeed was the case.

Figure 7 (plate 7) shows photomicrographs of corresponding portions of the internal surface of basi-occipital bones of + A and − A animals. It will be seen that, whereas in the + A animal there are abundant osteoclasts on the surface of the bone nearest the brain and placed directly in contact with the bone under the periosteum, there are no osteoclasts in the same position in the section from the − A animal. This point is shown more clearly in the semi-diagrammatic figure 8, plate 8 (of which figure 7 is a part). The section of bone in each case is a drawing, under camera lucida, but the osteoclasts seen in the histological preparation are marked in as black dots, in order to demonstrate their relative number and position. Here again it will be seen that the osteoclasts in some places are absent from, and in other parts greatly reduced on, the brain surface of the bone in the − A animal, but there are far more on the marrow surface of the same piece of bone than in the + A animal. In other words, the osteoclasts seem to have been reversed in position as compared with the + A animal.

A similar change in the position of osteoclasts has been observed in the vertebral bones of − A animals, an example of which is given in figure 9 (plate 9), which represents corresponding halves of two vertebrae of + A and − A dogs, and which is also of a semi-diagrammatic nature. Here again it will be seen that osteoclasts are few on the internal surface of the bone in the − A animal, whereas they are abundant in the same position in the + A animal. On the other hand, there are more osteoclasts on the marrow surface of the same piece of bone in the − A than

in the + A section. Here again there is a reversal of position of these cells. It may be added that in the original sections—both in the case of the basi-occipital bones (figure 8) and in that of the vertebrae (figure 9)—it can be seen that the osteoclasts on the nervous system side in the + A animals are active and are for the most part found in bone lacunae which they have obviously hollowed out. The evidence then shows that in the normal animal the increasing size of the central nervous system is accommodated by deposition on the outer surfaces and absorption on the internal surfaces of the surrounding bones. Apparently this mechanism breaks down in − A animals, because the osteoclasts are removed from the positions where they carry out this function of absorption to other positions on the bone. In their new positions they seem to be as numerous as, or even more numerous than, in the normal animal, and there is no question of their suppression. This explanation of the breakdown of the physiological growth fits the facts of the investigation as regards these bones, but it is not easy to understand. While lack of absorption of bone in the right place is the main feature in the occipital and vertebral bones of − A dogs and is undoubtedly responsible for many of the destructive changes of the nervous system described, it is not the only abnormality. There are obvious changes also in osteoblastic activity even in these bones. In figure 7, for instance, reversal of position of osteoblasts as well as osteoclasts can be seen. In some cases, as in the petrous bone, where there is laying down of extra bone, the increase in osteoblastic activity in − A dogs is much more prominent than that of changed absorption by osteoclasts.

The discovery that the position and activity of osteoblasts and osteoclasts are controlled by chemical means throws a new light on the physiology of bone growth, but there are many questions which must be answered before an understanding of this phenomenon can be obtained. Possibly the first question to be answered is whether vitamin A acts directly on bone cells or whether its effects are indirect, through some other humoral agency; the answer can probably be obtained by tissue culture experiments. It would be interesting also to know if, and how quickly, vitamin A and carotene can reverse the situation produced by their absence from the body.

It will be generally agreed that nature has done well to provide the central nervous system with a strong bony protection. Its safety from assault is essential both for the survival of the individual and the race. Those who build the walls and ramparts must, however, plan their activities in accordance with the size and growth of the citadel to be protected. The central nervous system is a citadel and as it grows the protecting walls are normally moved farther out. As the bones grow outward, their inner surface is removed to provide more space. Now if the director of building operations (vitamin A) disappears, it might then be expected that the working builders and demolition squad (osteoblasts and osteoclasts) would either cease to work or work in a completely disorderly way, but this does not happen in the bony ramparts. It is rather as if the place of vitamin A as a wise director of operations were taken over by that worst kind of director—the

energetic man with no wisdom, whom we all know so well nowadays—who says: 'I am going to show you how things should be done; now you will see something really happen.' His directions are: 'You must work harder than ever, but in a different way. You builders (osteoblasts) must lay down bricks wherever there is a foundation (periosteum). You demolishers (osteoclasts) working nearest the citadel must leave that position and continue your labours elsewhere.'

The result is that the walls, instead of covering a wider circumference as the citadel grows, now encroach on the nerve control stations, lines of communication and the administrative centres and squeeze all the vital structures into so small a space that work inside the citadel is impossible. Parts of the citadel (the central nervous system) are destroyed and the city (animal) with it. There has been no slacking and no anarchy among the building operatives but, by working in the wrong way, they have converted a protective structure into one of destruction. Thus vitamin A, by regulating the activities of the bone cells, coordinates a beautiful adjustment of bone and nervous system growth. How important this function is to animal development can be appreciated by the drastic and dramatic effects produced when the mechanism goes wrong in the absence of the vitamin.

In setting out the problem at the beginning of this lecture it was stated that the cause of the incoordination in young animals was independent of, but in some way related to, the cause of rickets. In the earlier stages of the investigation it was found that this incoordination was due to a deficiency of the vitamin A moiety of the original fat-soluble complex used in the rickets investigation and that the condition was associated with widespread degeneration of the central and peripheral nervous systems. It was thought at that time that the nerve degeneration was probably a direct reaction to the absence of vitamin A from the animal body. Later, however, it became clear that this was not the case, but that the degeneration was due to the fact that a function of the vitamin A moiety was to control the activity of osteoblasts and osteoclasts so that in its absence from the body the bones were thickened and altered in shape and that the incoordination was, in fact, due largely, if not entirely, to the pressure on the nervous system of this thickened bone. Rickets, on the other hand, is due primarily to a deficiency of the vitamin D moiety, whose main function is to control the deposition of a calcium-phosphate compound in the osteoid tissue laid down by the osteoblasts and thereby to harden the bones. Thus both these vitamins, working in close association, play an important part in bone growth—the one influencing the shape, the other the hardness. First vitamin A controls, or at least influences, the activity of the osteoblasts which lay down the soft bone. Vitamin D then attends to its calcification. Vitamin A again steps in and sees that any superfluous calcified bone is removed by osteoclastic action.

It is now clear how different and yet how closely related are these two problems of rickets and incoordination. Further investigation will no doubt show that the solution is not so simple as suggested and that other chemical agents play important parts in the scheme, but the present work does indicate that these two

inseparables, vitamins A and D, the David and Jonathan of nutrition, whose faithful alliance in natural distribution and in similarity of many chemical and physical properties has caused so much trouble to hosts of physiologists, biochemists, chemists and medical men, also work in harmony at the time of their active careers in the animal body. Although their functions are different, they are both concerned with the building up and maintenance of bone structure.

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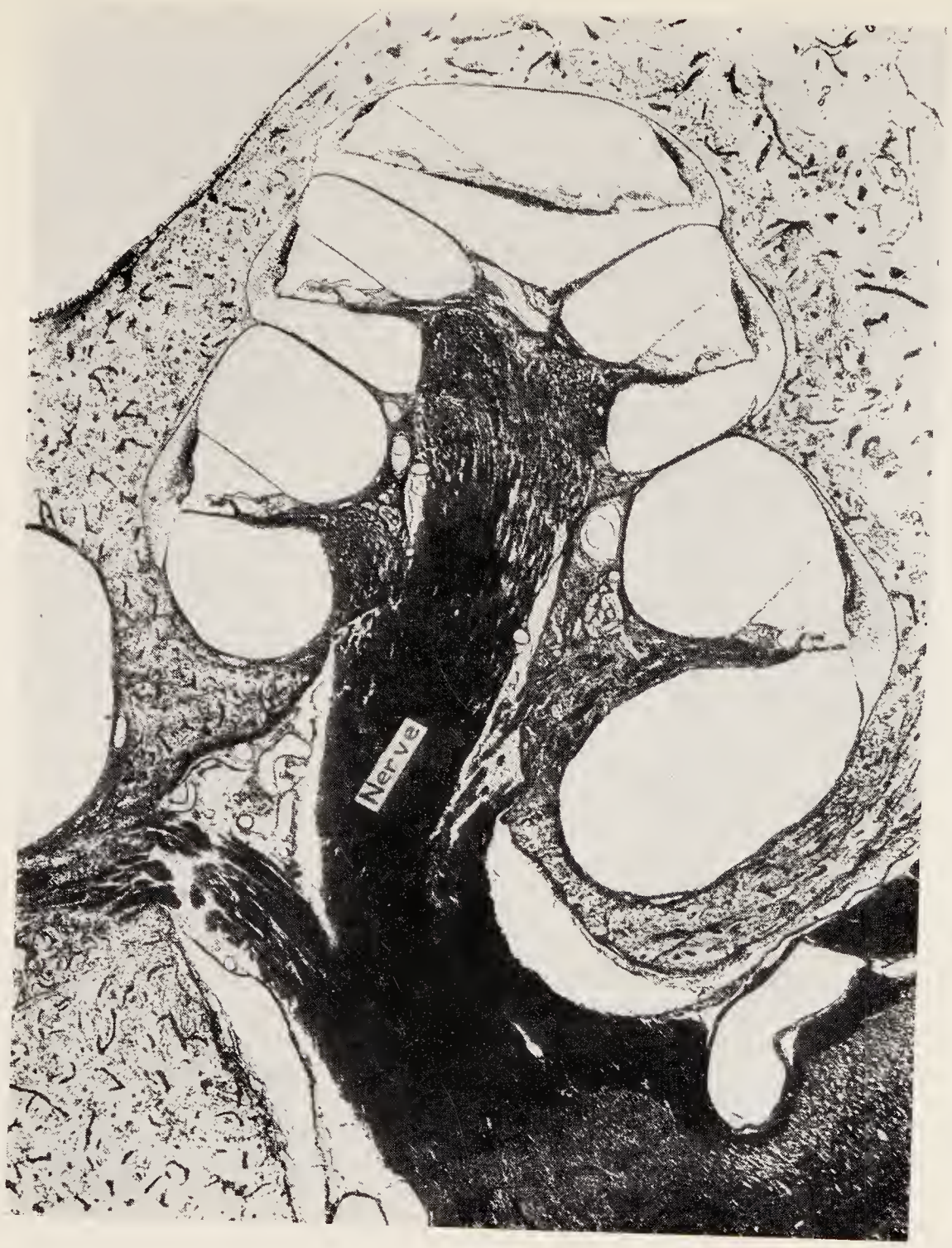

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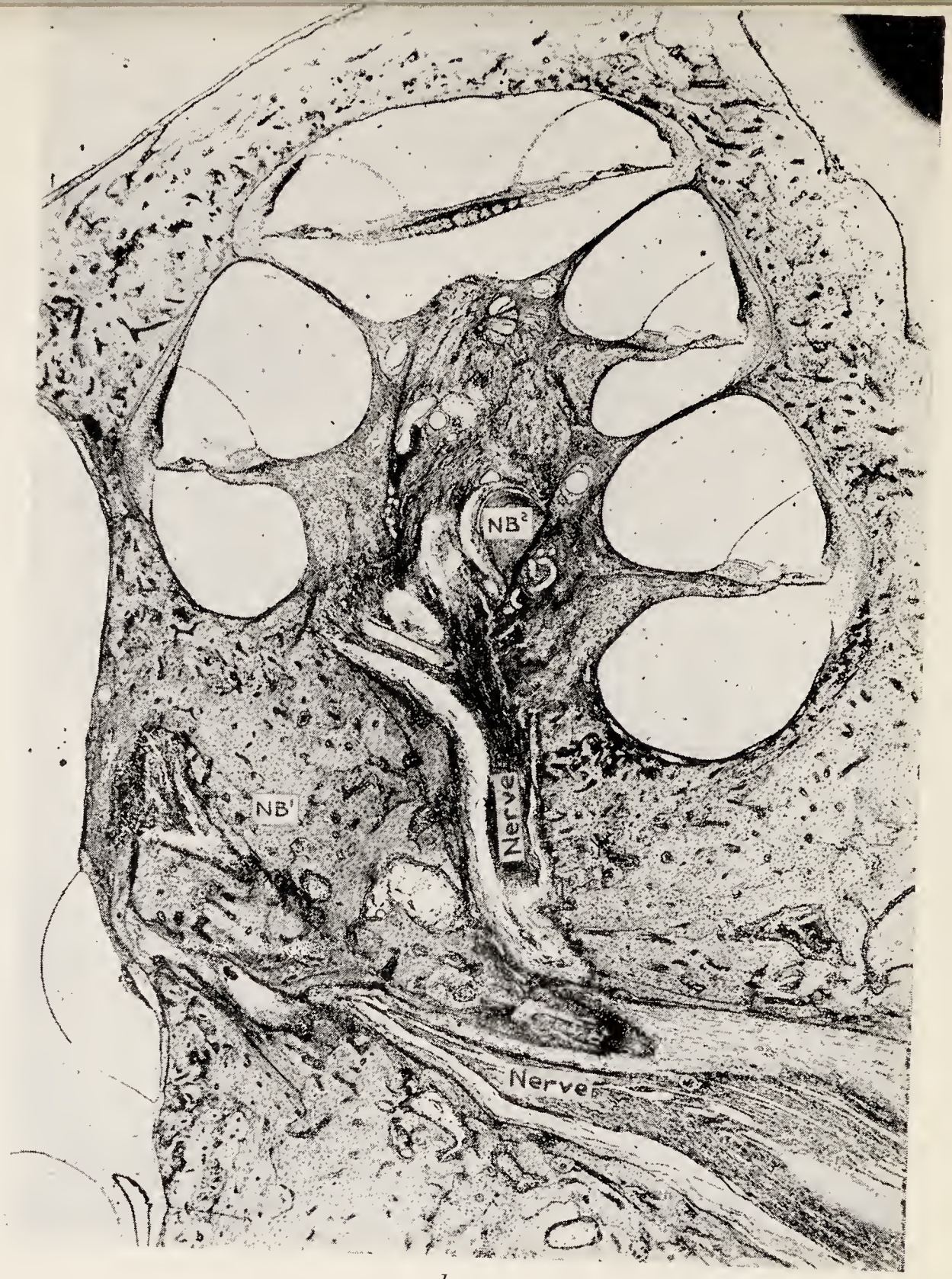

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FIGURE 1. Sections of cochleas and surrounding bones of +A (*a*) and −A (*b*) litter-mate dogs of the same age. *Note*. General narrowing of internal auditory meatus and compression of nerve by bone overgrowth (NB^1 and NB^2) in −A animal. The cochlea is situated farther from the brain in (*b*) than in (*a*).

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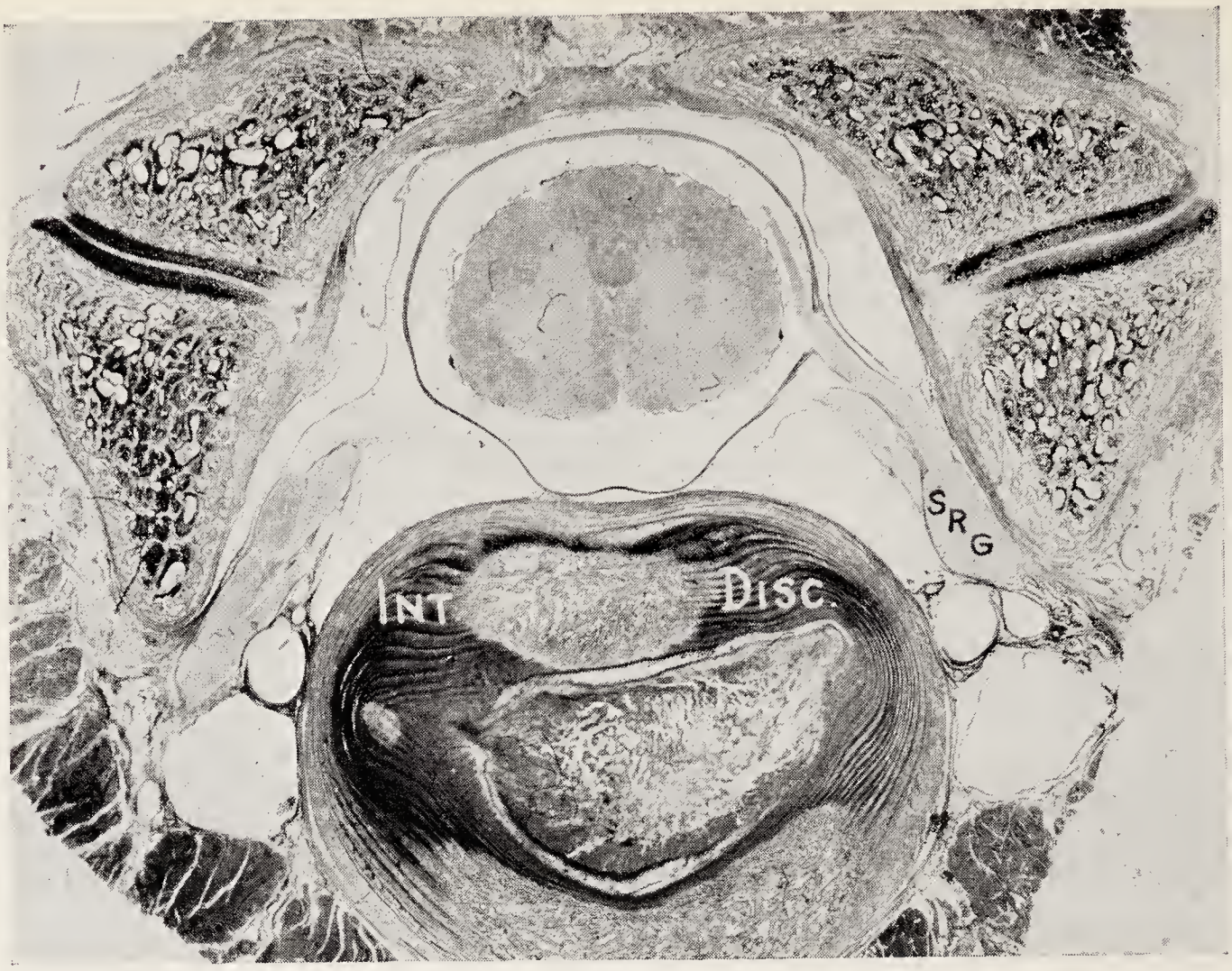

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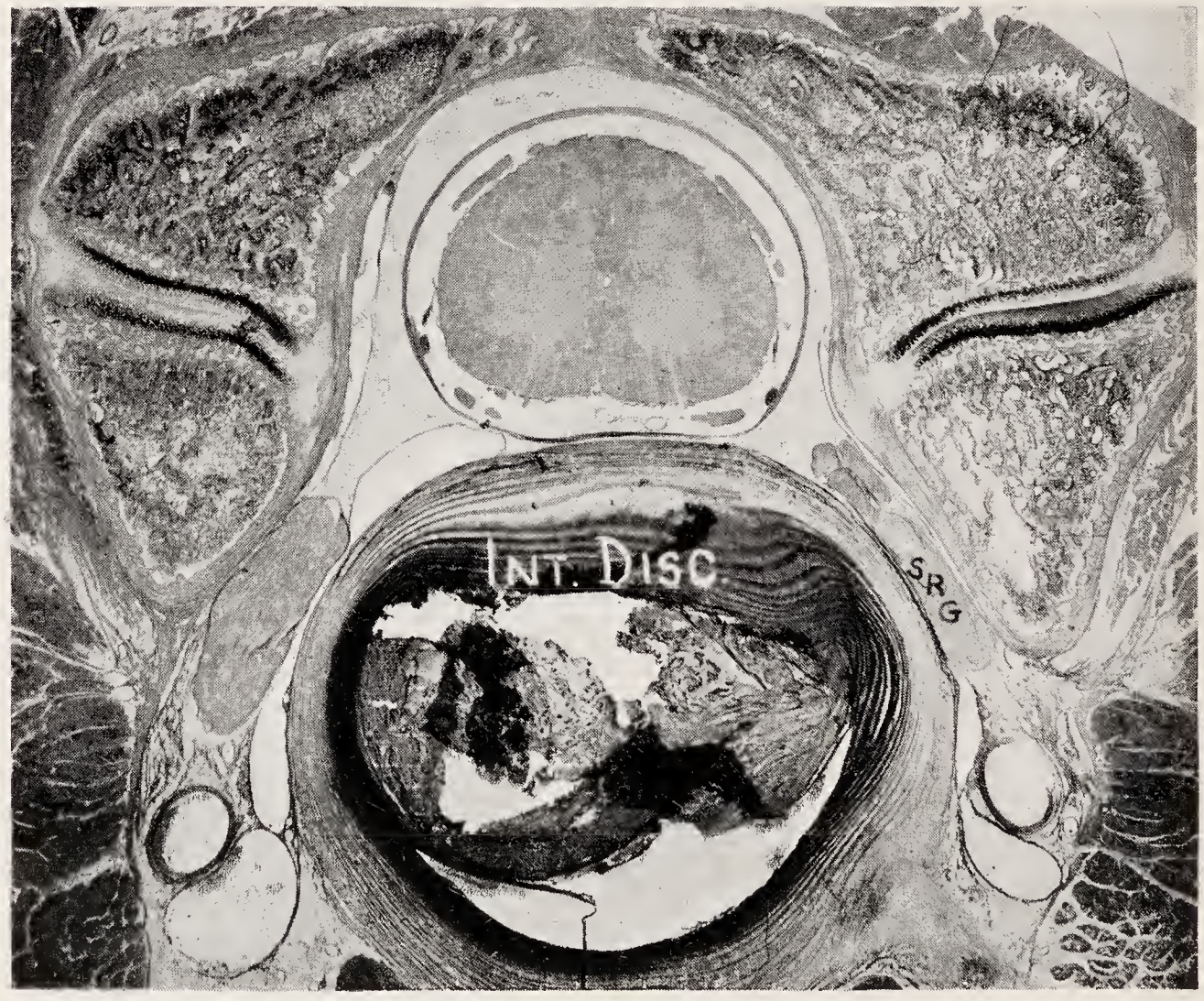

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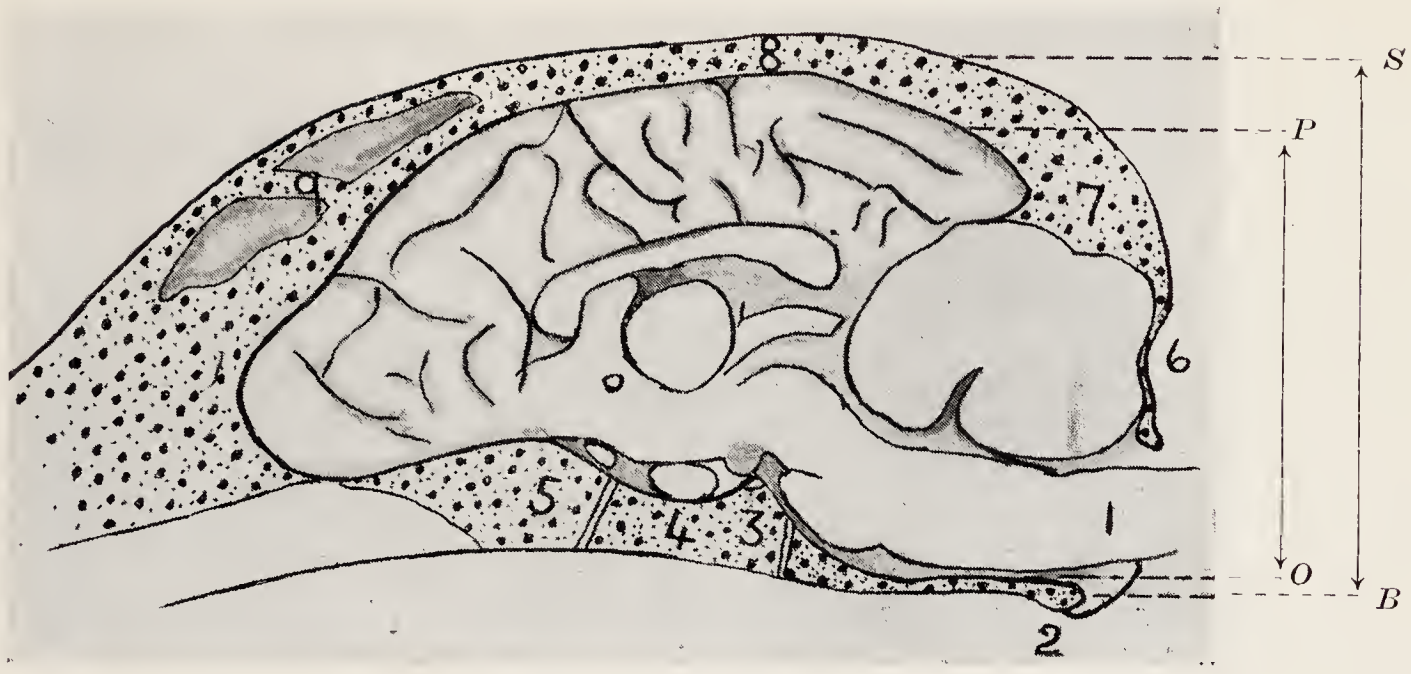
FIGURE 2. Transverse sections through the 5th cervical vertebrae and the spinal cord of +A (*a*) and −A (*b*) litter-mate dogs of the same age. *Note*. Reduction in size of the vertebral canal and the compression of the spinal root ganglion (*S.R.G.*) in the −A dog (*b*) as compared with the +A dog (*a*). There is less pressure on the left S.R.G. than on the right in (*b*).



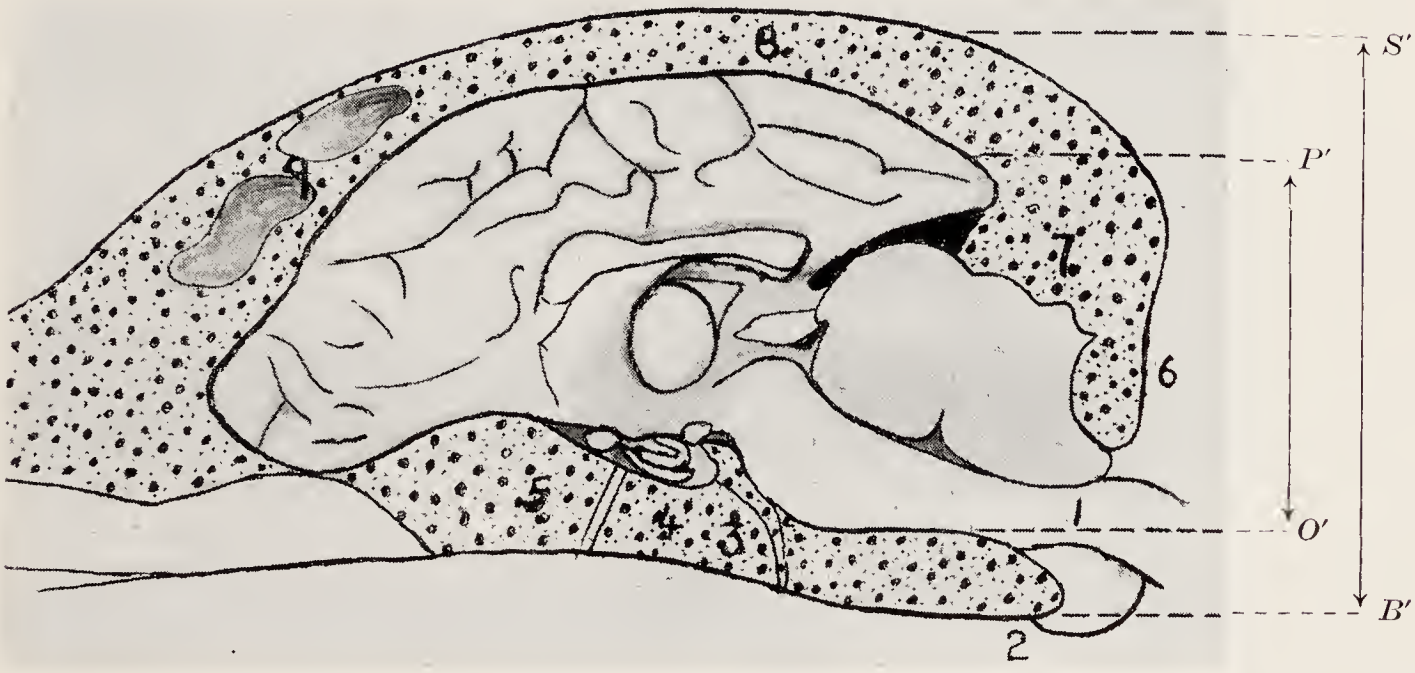


FIGURE 3. Drawings of mesial sagittal sections of skulls of +A (*a*) and −A (*b*) litter-mate dogs of the same age. (Bones stippled.) *Note.* (1) Great increase in thickness of bones surrounding the posterior fossa and to a less extent other bones in (*b*) as compared with those of (*a*). (2) Compression of medulla and cerebellum and pushing back of the posterior part of the cerebellum into the foramen magnum in (*b*). (3) Difference in measurements *PO*, *P′O′*, and similarity of *SB*, *S′B′*, showing the lack of absorption of bone on internal surface in (*b*). 1, foramen magnum; 2, basi-occipital; 3, posterior clinoid process; 4, basi-sphenoid; 5, anterior clinoid process; 6, supra-occipital; 7, occiput; 8, parietal; 9, frontal. The calcified tentorium cerebelli is marked in black in the −A animal (*b*).

a

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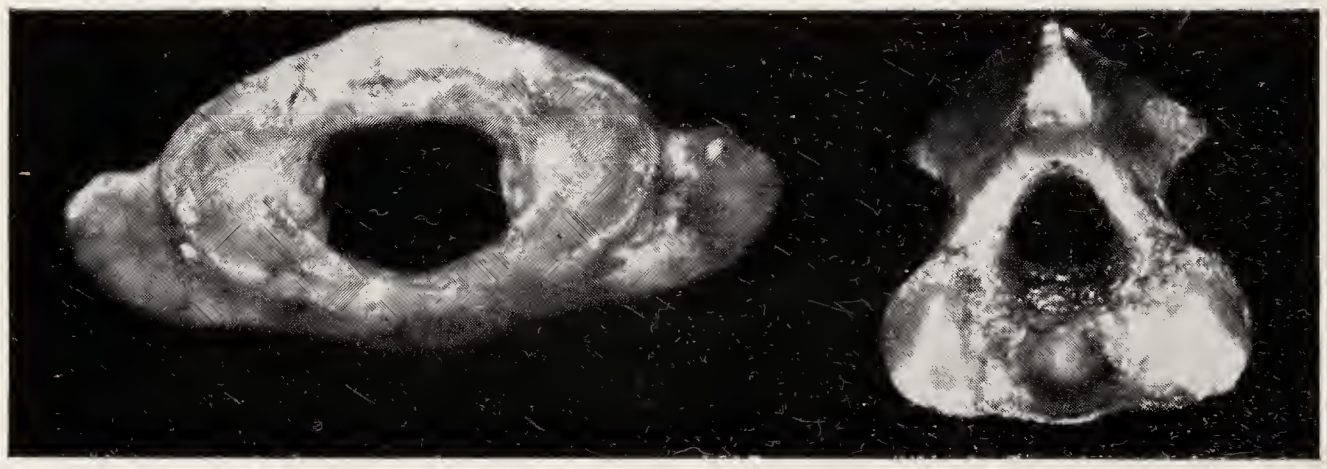

FIGURE 4. Atlas and axis vertebrae of +A (*a*) and −A (*b*) litter-mate dogs of the same age. *Note.* (1) The over-all size of the comparable vertebrae is not greatly dissimilar. (2) The vertebrae of the −A animal (*b*) are coarse and blunted and have lost their delicate outline. (3) The spinal canal in the vertebrae of the −A animal (*b*) is smaller than that of the +A animal (*a*).

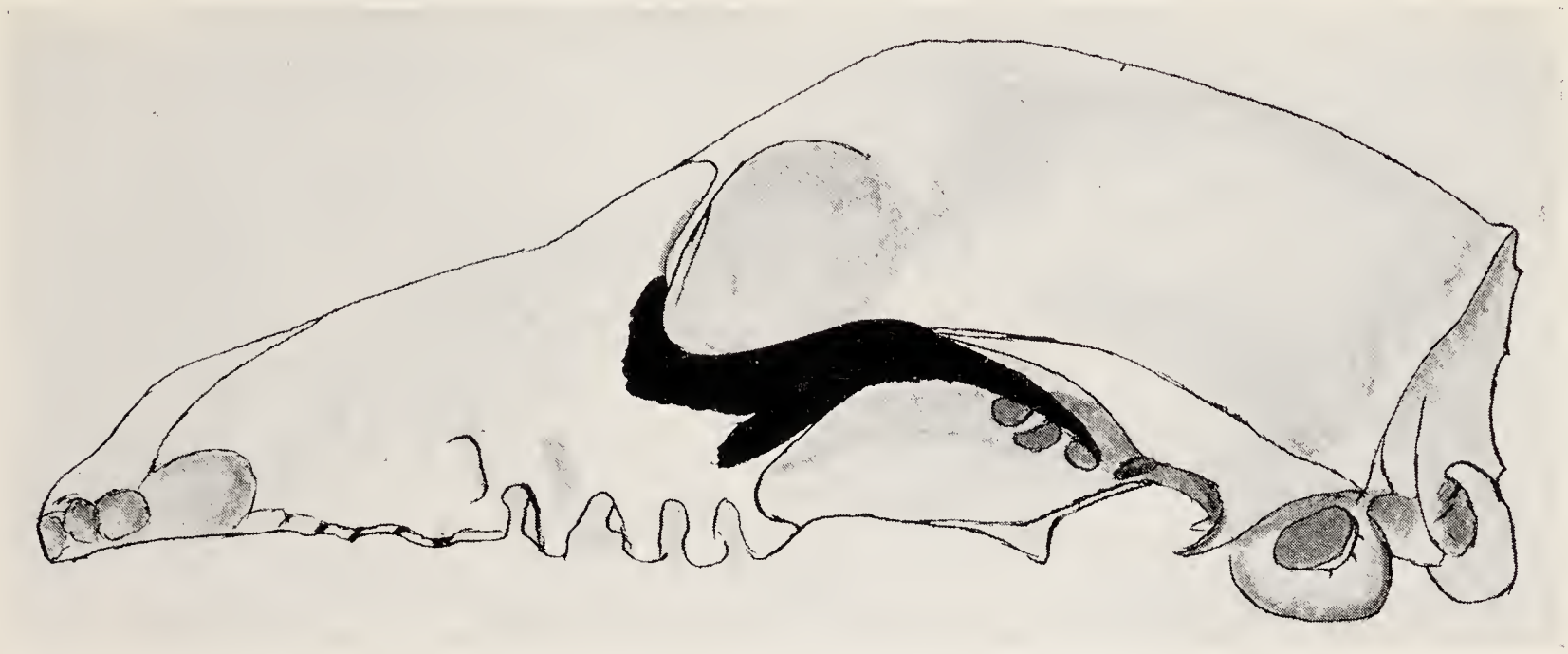

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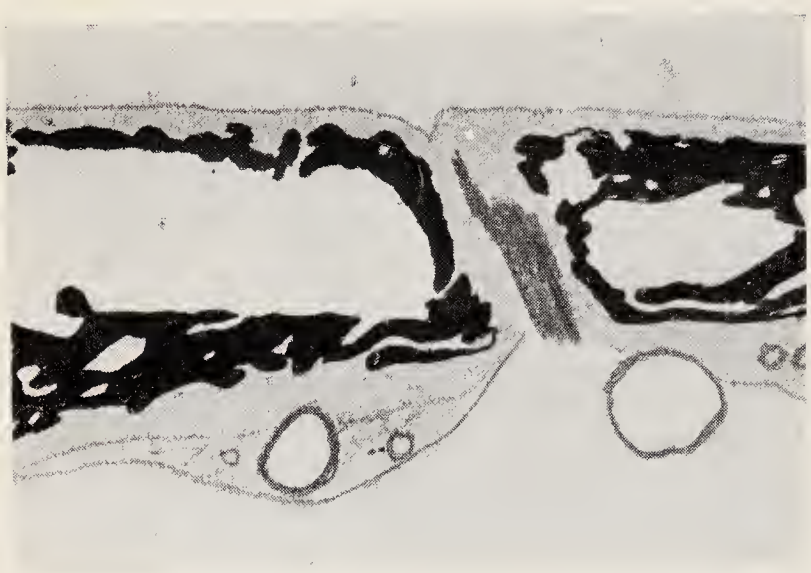
FIGURE 5. Drawings representing the lateral view of the skulls of +A (*a*) and −A (*b*) littermate dogs of the same age. The malar bone is black in each case. *Note* in the −A dog (*b*): (1) The increased size of the malar bone. (2) Its growth upwards into the lower margin of the orbit. (3) The general thickening of the zygomatic arch (malar bone and zygomatic process of temporal bone).



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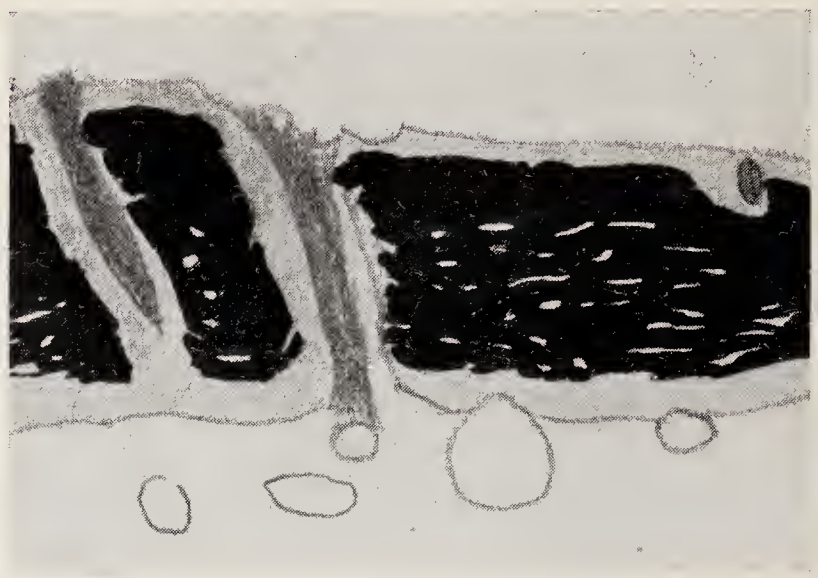

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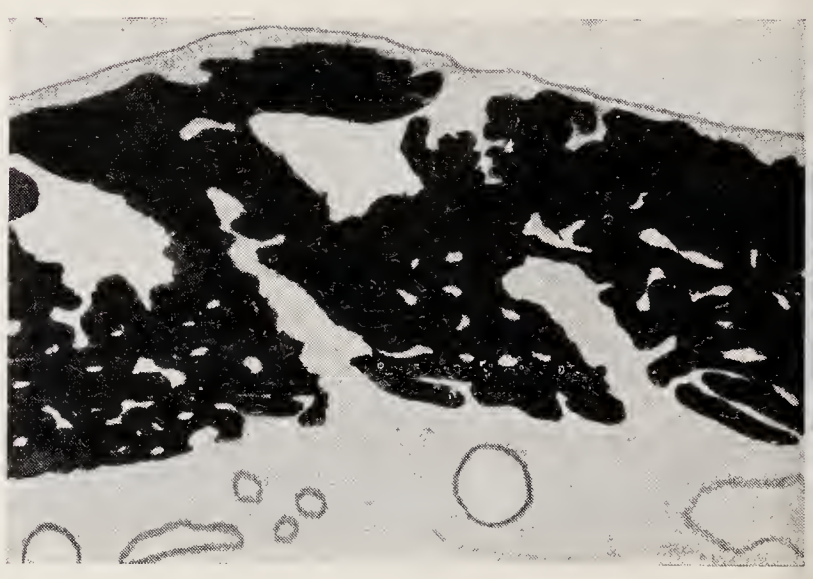

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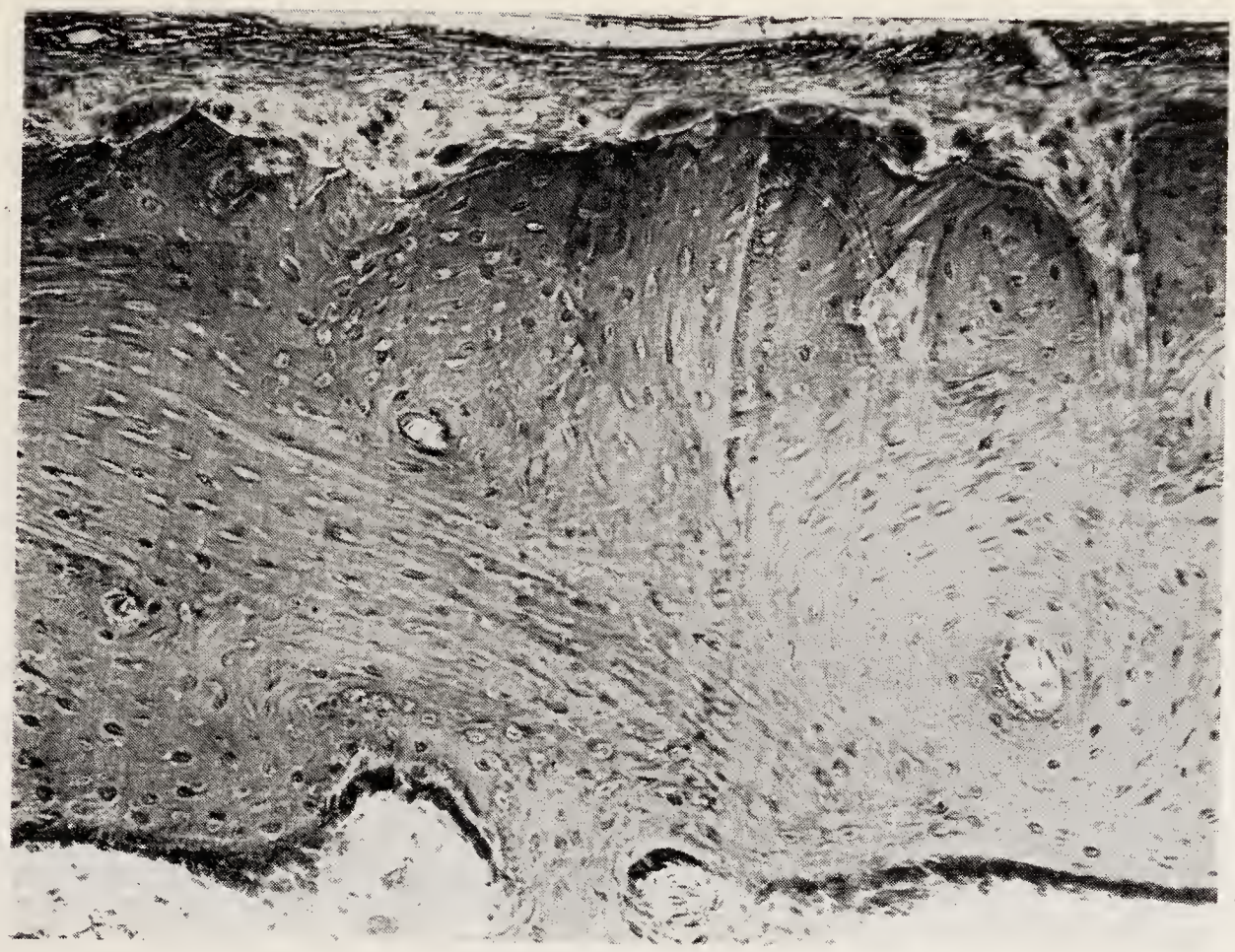
FIGURE 6. Drawings representing sections of parts of the cribriform plates of 4 litter-mate dogs of the same age, (*a*) +A, (*b*) −A, (*c*) +A+extra calcium (2 g. $CaCO_3$ daily), (*d*) −A+ extra calcium (2 g. $CaCO_3$ daily). The black areas indicate bone. *Note.* (1) A deficiency of vitamin A in the diet (with or without extra calcium) results in thickened plates. The addition of calcium does not prevent bone overgrowth. (Compare *a* with *b* and *c* with *d*.) (2) Addition of calcium (with or without vitamin A) increases the compact bone and reduces the cancellous bone. (Compare *b* with *d* and *a* with *c*.) (3) In the +A animals (*a* and *c*) there is ample space for the bundles of nerve fibres passing through the foramina of the cribriform plate. In the −A animals (*b* and *d*), however, the foramina are reduced in diameter and the nerves are squeezed.



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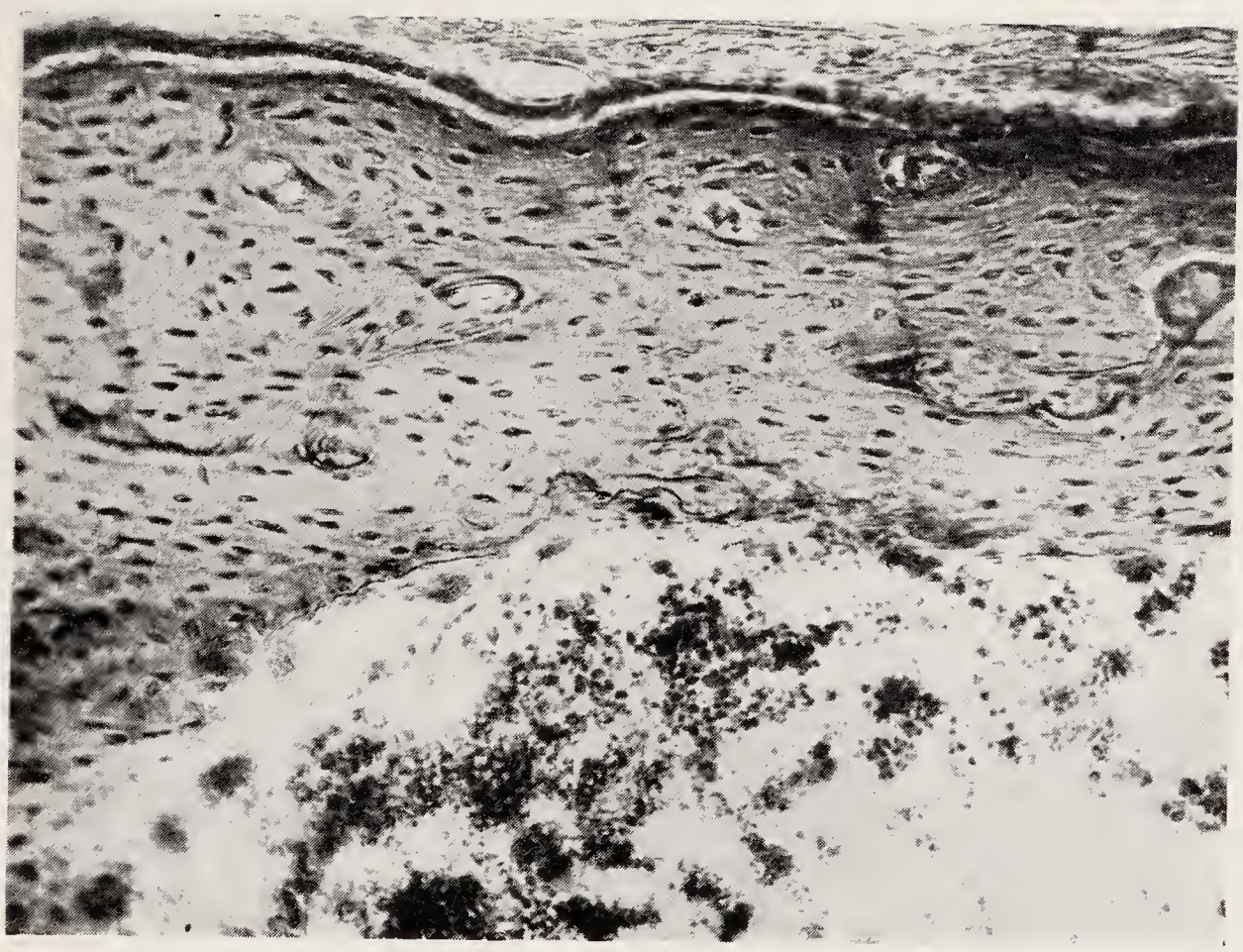

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FIGURE 7. Photomicrographs (× 135) of portions of the internal limiting plates of the basi-occipital bones of +A (*a*) and −A (*b*) litter-mate dogs of the same age. 1 indicates the bone surface nearest the brain; 2 indicates the marrow margin of the bone; *P* indicates periosteum. *Note*. Surface 1 (*a*): many osteoclasts but few osteoblasts; surface 1 (*b*): no osteoclasts but many osteoblasts; surface 2 (*a*): few osteoclasts but many osteoblasts; surface 2 (*b*): many osteoclasts but few osteoblasts.

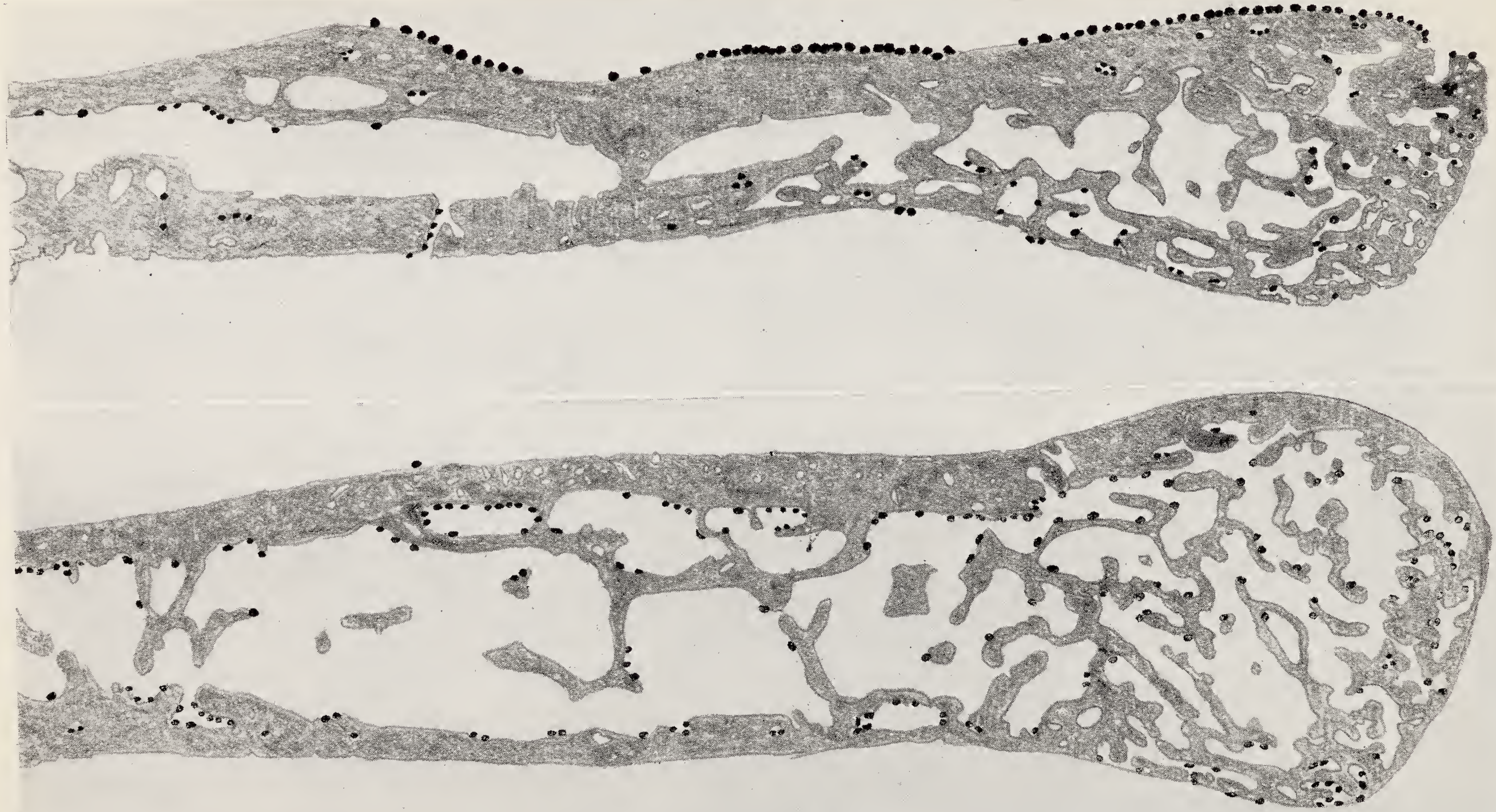

FIGURE 8. Semi-diagrammatic drawings representing the basi-occipital bone in +A (*a*) and −A (*b*) litter-mate dogs of the same age. The osteoclasts in each case are indicated by black dots. *Note.* (1) Thickening of the bone in the −A animal (*b*). (2) In the +A animal (*a*), there is a large number of osteoclasts on the surface of the bone adjacent to the brain (upper surface in diagram). In the −A animal (*b*) they are absent from this region but are abundant on the marrow surface of the same portion of bone. As in the case of figure 7, there seems to have

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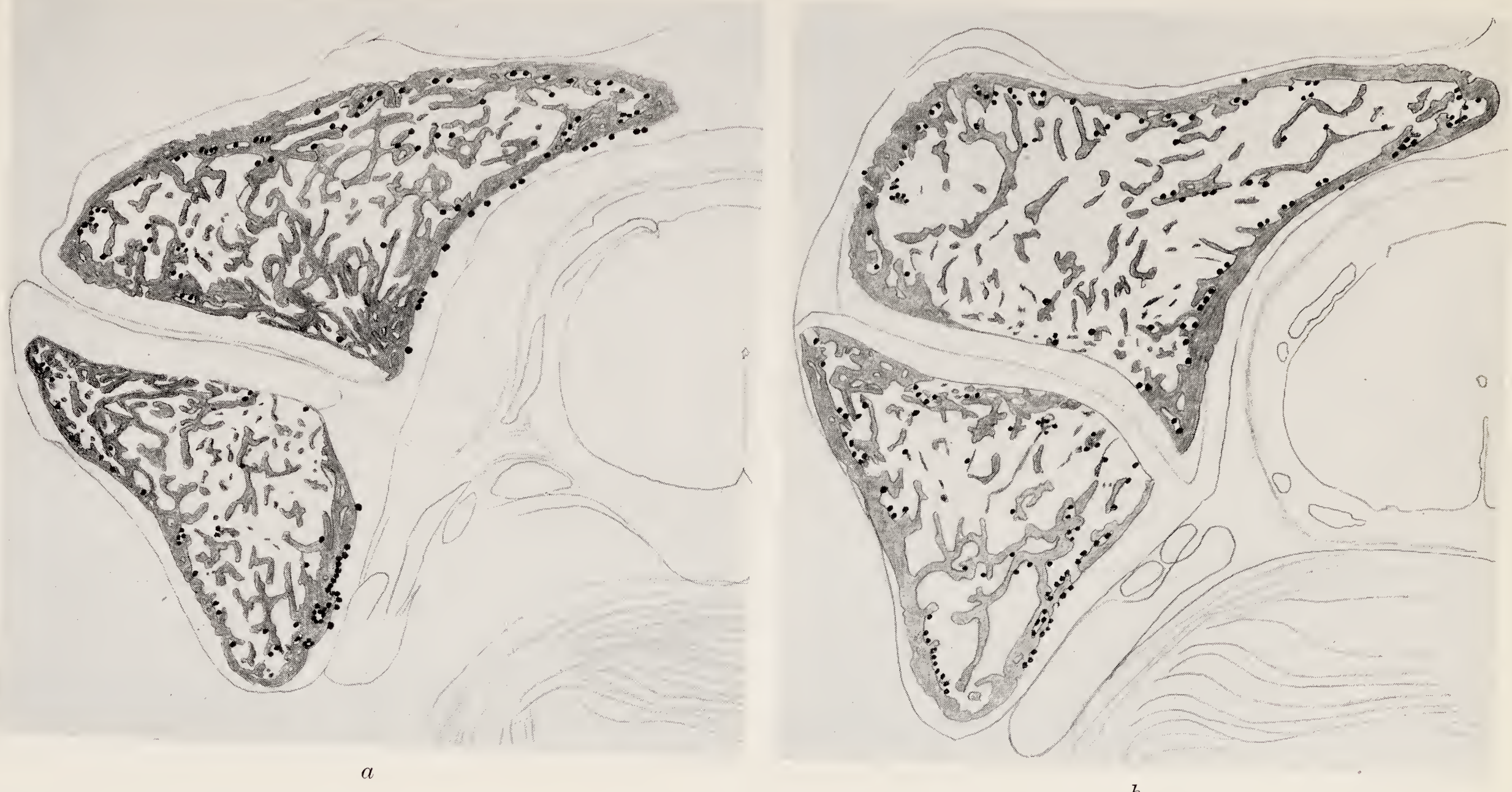

a *b*

FIGURE 9. Semi-diagrammatic drawings representing the lateral portions of comparable vertebrae of +A (*a*) and −A (*b*) litter-mate dogs of the same age. The osteoclasts in each case are indicated by black dots. *Note.* (1) Enlargement of the bones in the −A animal (*b*). (2) In the +A animal (*a*) the osteoclasts on the surface of the bone adjacent to the spinal cord are numerous. In the −A animal (*b*) relatively few osteoclasts are found in this position. On the marrow side of this same portion of bone (*b*), however, they are more numerous than in the corresponding position in the +A animal (*a*). There seems, in fact, as in figures 7 and 8, to have been a reversal of position of the osteoclasts in this region in the −A animal.